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Plants as Sources of Antimalarial Drugs: In Vitro Antimalarial Activities of Some Quassinoids

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Fourteen quassinoids, obtained from simaroubaceous plants, were tested for in vitro antimalarial activity. All of these inhibited the incorporation of [^{3}H]hypoxanthine into *Plasmodium falciparum* in vitro at concentrations below 0.41 μg ml^{-1}. The two most potent quassinoids, bruceantin and simalikalactone D, showed 50% inhibitory concentration values of 0.0008 and 0.0009 μg ml^{-1}, respectively. The results are compared with the antiamoebic, antileukemic, and cytotoxic activities of these compounds reported in the literature.

In the 1960s, the appearance in Southeast Asia and South America of strains of *Plasmodium falciparum* showing resistance to chloroquine heralded the need for alternative antiplasmodial therapy. Several hundreds of millions of people suffer from malaria, and all currently used antimalarial drugs now show limitations in their spectra of activity (17). Resistance of *P. falciparum* to available antimalarial drugs is an increasing world problem (10). Therefore, it is crucial that mechanistically novel antimalarial agents be added to our chemotherapeutic armamentarium as soon as possible.

Certain quassinoids, obtained from simaroubaceous plants, are known to possess a variety of biological activities, including antitumor (13), antiviral (11), antifeedant (12), antiamoebic (5), and antiinflammatory (7) activities. More recently, some of these compounds, namely, bruceantin (6), simalikalactone D (16), glaucarubinone (16), soularubinone (16), and sergeolide (4), have been found to show high activity against *P. falciparum* in vitro. Sergeolide also markedly reduces virulence of experimentally induced *P. berghei* in mice (4); however, it unfortunately also shows high toxicity. In our present study, we monitored the in vitro anti-*P. falciparum* activities of a series of 14 quassinoids which were made available from isolations performed under the auspices of the National Cancer Institute, Bethesda, Md. Our results are considered in the light of published data for other biological activities of these quassinoids.

(This report is part 2 of a study on plants as sources of antimalarial drugs. For part 1, see reference 9.)

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MATERIALS AND METHODS

***P. falciparum* strain.** A chloroquine-resistant strain (K-1) of *P. falciparum*, which was originally obtained from Thailand (14) and has been cryopreserved at the London School of Hygiene and Tropical Medicine, was used throughout.

Maintenance of cultures. Cultures of *P. falciparum* were maintained in vitro in human blood cells (O+ve) diluted to 5% hematocrit with RPMI 1640 medium (10% human O+ serum) by published techniques (2, 3, 15).

Test protocol. The test procedure was based upon the method of Desjardins et al. (2). The quassinoids were dissolved in ethanol and diluted with RPMI 1640 medium before testing. The concentration of ethanol in the test never exceeded 0.1%, and controls demonstrated that there was no effect on [^{3}H]hypoxanthine incorporation. Portions (50 μl each) of diluted quassinoids were dispensed into 96-well microtiter trays so as to yield final test concentrations of 50, 5, 0.5, 0.05, 0.005, and 0.0005 μg ml^{-1}. More accurate determination of 50% inhibitory concentration (IC_{50}) values was achieved with 12 2-fold dilutions at concentrations around the range of the value obtained by 10-fold dilutions. All tests were performed in duplicate. To each well was added 50 μl of human erythrocytes (O+ve, diluted to 5% hematocrit) with 1% parasitemia (dilutions to 1% parasitemia were made with uninfected washed erythrocytes). Two series of controls were performed, one with parasitized blood without quassinoid and another with uninfected erythrocytes without quassinoid. The IC_{50} value for chloroquine was determined during each experiment. After incubation in a 3% O_2–4% CO_2–93% N_2 gas phase for 18 h at 37°C, 5 μl of [G-^{3}H]hypoxanthine (40 μCi ml^{-1}; Amersham Corp., United Kingdom) was added to each well, and incubation was continued for a further 18 to 24 h.

Harvesting. Erythrocytes were washed from the wells with normal saline with a Titertek cell harvester (Flow Laboratories, Inc., McLean, Va.) through a glass fiber membrane predampened with saline. The glass fiber membrane was flushed with distilled water for 20 s to lyse erythrocytes and then flushed with saline for 20 s to remove remaining traces of hemoglobin. After being washed further with distilled water and saline (20 s with each), the membrane was dried, and the glass fiber disc for each well was pushed out into polypropylene scintillation vials (4-ml volume). To each vial, 4 ml of scintillation fluid (Packard toluene scintillator) was added, and the counts per minute were determined for 10 min at about 30% efficiency.

Analysis of results. Counts per minute were converted to disintegrations per minute by using an external standard, and the percentage of inhibition was calculated from the following equation: percent inhibition = 100 − {[(disintegrations

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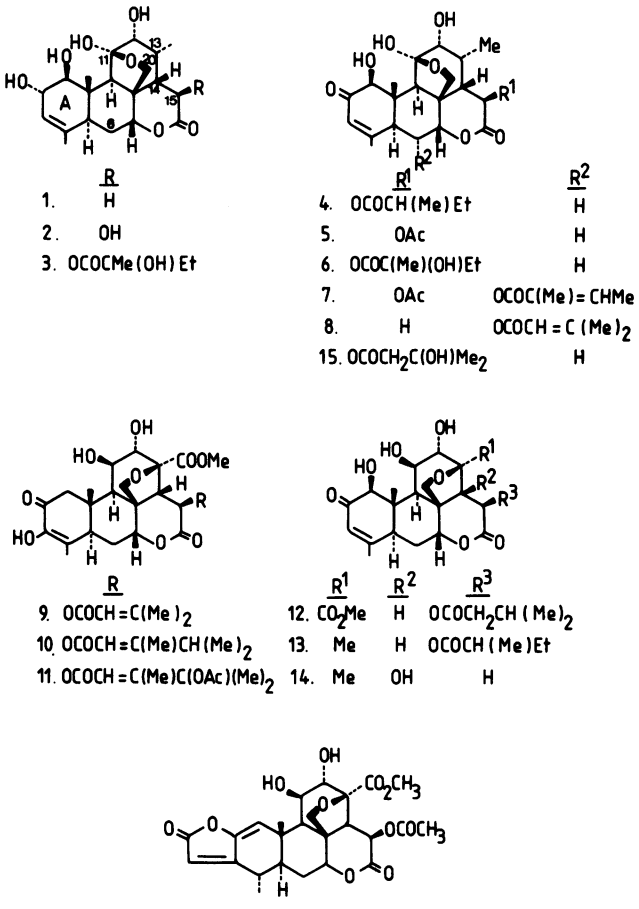

FIG. 1. Structures of quassinoids. 1, Chaparrin; 2, glaucarubol; 3, glaucarubin; 4, ailanthinone; 5, holacanthone; 6, glaucarubinone; 7, undulatone; 8, 6α-senecioyloxychaparrinone; 9, brusatol; 10, bruceantin; 11, bruceantinol; 12, isobruceine A; 13, simalikalactone D; 14, samaderine E; 15, soularubinone; 16, sergeolide.

per minute in infected erythrocytes plus quassinoid) − disintegrations per minute in uninfected erythrocytes] × 100}/(disintegrations per minute in infected erythrocytes − disintegrations per minute in uninfected erythrocytes). Concentration-versus-percent inhibition curves were interpreted by linear regression analysis, from which the IC_{50} values and their 95% confidence intervals were determined.

Sources of quassinoids. The compounds used in the study were isolated from species of the family *Simaroubaceae* and were obtained from the repository of the National Cancer Institute, Bethesda, Md. (Fig. 1, compounds 1 to 14).

RESULTS

The results are presented in Table 1. Of the 14 quassinoids tested, all showed in vitro antimalarial activity, having IC_{50} values below 0.41 μg ml^{-1}. Of these quassinoids, 10, from ailanthinone to simalikalactone D (Table 1), possessed IC_{50} values of less than 0.02 μg ml^{-1}. Chloroquine diphosphate showed an IC_{50} value of 0.21 μg ml^{-1} in the same test.

DISCUSSION

The in vitro antimalarial activities in our study for simalikalactone D (IC_{50}, 0.0009 μg ml^{-1}) and glaucarubinone (IC_{50}, 0.004 μg ml^{-1}) are of the same order as those reported by Trager and Polonsky (16), namely, 0.002 μg ml^{-1} for simalikalactone D and 0.006 μg ml^{-1} for glaucarubinone. The results reported in Table 1 show the importance for activity of the presence of an ester function at C-15; glaucarubin is about three times more active than chaparrin and about eight times more active than glaucarubol. Changes in the nature of this ester function produce marked alterations in activity; glaucarubinone is about twice as potent as holacanthone, whereas bruceantin is more than twice as active as bruceantinol and more than three times as active as brusatol. A comparison of the activities of glaucarubinone and 6α-senecioyloxychaparrinone suggests that an ester function at C-15 improves activity over one at C-6. Also, if the C-15 is already esterified, additional esterification at C-6 appears to offer little enhancement in activity, as shown by the IC_{50} values of holacanthone and undulatone. The A-ring substitution pattern is apparently crucial to activity; glaucarubinone, having an α,β-unsaturated keto function in ring A, is over 10 times more active than glaucarubin. There is no obvious overall difference in activities of compounds with a C-20 to C-11 oxygen bridge or a C-20 to C-13 oxygen bridge.

The in vitro antimalarial activities of the quassinoids in the present study did not always parallel their other biological activities, as reported in the literature. Table 1 lists reported data for the antileukemic, antiamoebic, and cytotoxic activities of some of the quassinoids tested for in vitro antimalarial activity in this study. Trager and Polonsky (16) reported that the in vitro antimalarial activities of the five quassinoids they tested (viz., simalikalactone D, glaucarubinone, soularubinone, simarolide, and chaparrinone) parallelled their antileukemic activities. In our study, the in vitro antimalarial activities of the quassinoids did not exactly parallel their in vivo murine lymphocyte leukemia (P-388) optimal test-to-control survival values or optimal doses reported by Cassady and Suffness (1) and Suffness (unpublished data). However, the compounds with the highest in vitro antimalarial activity in our test, bruceantin (IC_{50}, 0.0008 μg ml^{-1}) and simalikalactone D (IC_{50}, 0.0009 μg ml^{-1}), rate among the best antileukemic quassinoids reported (Table 1).

Bruceantin and simalikalactone D were also found (5) to possess the highest in vitro amoebicidal activity of a series of 17 quassinoids examined. Bruceantin had an IC_{50} of 0.018 μg ml^{-1}, and simalikalactone D had an IC_{50} of 0.047 μg ml^{-1} in the latter test. Apart from this, there is little similarity between in vitro antimalarial and antiamoebic activities of the quassinoids; glaucarubinone was about twice as active as ailanthinone in the antimalarial test but only half as active as ailanthinone in the antiamoebic test. Also, glaucarubin, which has only relatively modest in vitro antimalarial activity (IC_{50}, 0.055 μg ml^{-1}), was active in the antiamoebic test (IC_{50}, 1.57 μg ml^{-1}), whereas some quassinoids with high in vitro antimalarial activities, viz., holacanthone, undulatone, 6α-senecioyloxychaparrinone, brusatol, bruceantinol, and samaderine E, were not active in the antiamoebic test at 2 μg ml^{-1}, the highest concentration tested. It is worth noting that chloroquine sulfate (antiamoebic IC_{50}, 85 μg ml^{-1}) also displayed little antiamoebic activity.

Some quassinoids are known to be toxic to mammalian cells (1), and, in the present study, 6α-senecioyloxychaparrinone, bruceantin, and simalikalactone D proved to be the most potent quassinoids tested against 9KB cells (Table 1). Apart from ailanthinone, 6α-senecioyloxychaparrinone, and simalikalactone D, all of the quassinoids tested were active

TABLE 1. In vitro antimalarial activities of quassinoids compared with their in vitro cytotoxic and antiamoebic and in vivo antileukemic activities

Compound	Antimalarial activity in vitro		Cytotoxic activity in vitro (ED_{50} for 9KB cells [$\mu g\ ml^{-1}$])[b]	Antiamoebic activity in vitro (IC_{50} [$\mu g\ ml^{-1}$])[c]	Antileukemic activity in vivo (P-388)	
	IC_{50} ($\mu g\ ml^{-1}$)[a]	95% Confidence interval			Optimal test-to-control survival (%)[d]	Optimal dose (mg kg of body wt^{-1}) $injection^{-1}$[e]
Chaparrin	0.180	0.092–0.352	6.5	−ve	145	40
Glaucarubol	0.410	0.132–1.271	5.5	−ve	100	25
Glaucarubin	0.055	0.030–0.101	5.1	1.57	127	2.0
Ailanthinone	0.009	0.005–0.018	0.03	0.068	148	2.0
Holacanthone	0.007	0.004–0.013	0.20	−ve	190	4.0
Glaucarubinone	0.004	0.002–0.007	0.04	0.14	177	0.25
Undulatone	0.006	0.003–0.012	0.30	−ve	163	5.0
6α-Senecioyloxychaparrinone	0.008	0.004–0.016	0.007	−ve	198	1.0
Brusatol	0.003	0.002–0.005	0.11	−ve	104	0.5
Bruceantin	0.0008	0.0004–0.002	0.007[f]	0.018	220	0.5
Bruceantinol	0.002	0.001–0.003	0.02	−ve	238	1.0
Isobruceine A	0.002	0.001–0.004	0.02	NT[g]	163	2.0
Simalikalactone D	0.0009	0.0004–0.002	0.003	0.047	198	1.0
Samaderine E	0.015	0.008–0.033	NT	−ve	156	0.5
Chloroquine diphosphate	0.210	0.190–0.240	14.0	85[h]		

[a] Inhibition of uptake of [^{3}H]hypoxanthine into *P. falciparum* in vitro; IC_{50} based on twofold dilutions done in duplicate.
[b] Values are those of Cassady and Suffness (1). Data for chaparrin, glaucarubol, glaucarubin, brusatol, bruceantin, and chloroquine diphosphate are from M. Suffness (unpublished observations). 9KB cells are from human epidermoid carcinoma of the mouth. ED_{50}, Dose required to reduce growth (protein content) to 50% that of controls. These values indicate the cytotoxic activity in vitro.
[c] Values are those of Gillin et al. (5) with *Entamoeba histolytica* HM-1 IMSS, −ve. Quassinoid inactive at 2 $\mu g\ ml^{-1}$. These values indicate the antiamoebic activity in vitro.
[d] Values are those of Cassady and Suffness (1).
[e] Values are those of M. Suffness (unpublished observations).
[f] Not as previously reported (1).
[g] NT, Not tested.
[h] Values are those of Neal (8) for chloroquine sulfate.

in the in vitro antimalarial test at concentrations well below their toxic concentrations against 9KB human tumor cells in vitro. In both tests, the presence of an α,β-unsaturated keto function in ring A is of overriding importance for activity; chaparrin, glaucarubol, and glaucarubin are about 20 times less cytotoxic in the 9KB cell test than undulatone, the least toxic of the compounds, which has an α,β-unsaturated keto group in ring A. However, apart from this similarity, in vitro antimalarial activity does not parallel cytotoxicity against 9KB cells. In the first place, a C-15 ester function, which is important for antimalarial activity, is of less significance to cytotoxicity in the 9KB cell test. Chaparrin, glaucarubol, and glaucarubin, which have markedly different activities in the antimalarial test, show very similar toxicities against 9KB cells. However, when a C-15 ester group is present, changes in the nature of this ester function produce distinct alterations in the activities in both tests. Different criteria for the ester function appear to operate in the two tests. For antimalarial activity, the 2-hydroxy-2-methylbutyric ester at C-15, as in glaucarubinone, is about twice as active as the C-15 acetate as in holacanthone, whereas glaucarubinone is about five times more active than holacanthone against 9KB cells. Also, bruceantin, containing a 3,4-dimethyl-2-pentenoic chain at C-15, is over 3 times more active than brusatol (senecioic moiety at C-15) in the antimalarial test but is over 10 times more toxic than brusatol against 9KB cells. Another difference in structural requirements for activity in the two tests is illustrated by comparing the activities of glaucarubinone, which is esterified at C-15, and 6α-senecioyloxychaparrinone, which is esterified at C-6; glaucarubinone is about twice as active as 6α-senecioyloxychaparrinone in the antimalarial test but is almost five times less active against 9KB cells. These important differences in the activities of compounds in the two tests suggest that it may be possible to find a quassinoid with good antimalarial activity and low mammalian cytotoxicity.

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